Shell shape variation within a population of *Astarte borealis* (Schumacher, 1817) (Bivalvia: Astartidae) from Camden Bay, northern Alaska: a study using elliptical Fourier analysis

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ABSTRACT

Shells in the bivalve genus *Astarte* are known for variable morphology and polymorphism within living and fossil species. *Astarte borealis* (Schumacher, 1817), the most common living species, is recognizable and common in the mid- to high-latitude North Pacific, Arctic, and North Atlantic oceans, and has been previously subdivided into several subspecies and varieties based on variations in overall shell shape. A collection of 641 recent specimens of *A. borealis* from Camden Bay, northern Alaska, with intact outlines was analyzed for variability of the shell shape within a population of the species. The analysis has important implications for morphological studies of recent and fossil bivalve mollusks. Bivariate analysis of length vs. height and morphometric analysis of shell outline determined variants within a population of *A. borealis*, and were compared to Pliocene *A. borealis*. The computer program SHAPE version 1.3 (Iwata and Ukai, 2002) uses elliptic Fourier coefficients of shell outlines to evaluate and visualize shape variations. The multivariate outline analysis indicates that intraspecific shell variation in *A. borealis* is based upon a modal shape that grades into other shapes, rather than grade between two or more end-forms.

Additional Keywords: Multivariate outline analysis

INTRODUCTION

It has long been suggested that the outlines of objects, mollusk shells in this case, are of great significance to visual recognition and are therefore important for classification purposes (Scott, 1980). The outline measurements, however has not been widely used in taxonomic studies, in favor of more traditional distance measurements of height, length, and width, and relative geometric locations of certain well-defined characters, known as "landmarks". Part of the problem was the lack of suitable instrumentation for precise outline measurements. Advances in computer technology and digital image analysis have taken outline data collection and processing to a new level. Computer-based techniques of shell shape quantification are particularly important for fossil species, where other diagnostic characteristics, such as those in soft tissues, are unavailable for study. One of the common problems arising from attempts to identify large number of similarly shaped individuals is the recognition of patterns of variability within the population(s) that can be used as a basis for precise taxonomic identification. As a test case for outline shape analysis, we selected the high-latitude bivalve *Astarte borealis*. Recent and Pliocene specimens from Alaska were evaluated. Bivalves of the genus *Astarte* Sowerby, 1816 are also notorious for the conservatism in shell shape that made them particularly suitable for this study (Dall, 1920).

The bivalve genus *Astarte* is known from as early as the Lower Jurassic in northern Siberia (Zakharov, 1970). Recent species of *Astarte* are common in circumpolar panarctic waters and are known for polymorphism (Zettler, 2001). One of the most abundant recent species is *Astarte borealis* (Schumacher, 1817). The species is known to have a high degree of shell shape variability and has been often been called a "species complex", by various authors owing to numerous subspecies and named varieties (Zettler, 2001, 2002; Ockelmann, 1958; Petersen, 2001). Although there are several synonyms attributed to this polymorphic species, there have been few detailed morphological studies done. Qualitatively, the shell varies from ovate to subtrigonal and quadrangular within the genus. Among other characteristics, these qualitative terms are often found in the descriptions of *Astarte* species (Dall, 1903, 1920; Coan et al., 2000). A more precise definition of the shell shape is necessary in an attempt to better quantify the shape of the shell. One of the ways to examine the amount of variation and evaluate the possible presence of different shape morphs with gradation, for both recent and fossil *Astarte* species, is to use statistical techniques to study shell shape variability.

The objective of this study was to quantitatively determine the degree of morphological variability in shell shape within a population of recent *Astarte borealis*, from a single location in Arctic Alaska, based on a large sample size. The hypothesis for this study is that a population of extant *A. borealis* in Camden Bay, northern Alaska, shows a high degree of variation in shell outline morphology. The variation is attributed to a central form grading into a range of variants rather than the presence of two or more distinct forms. An unbiased clustering around the central form will indicate that any separation of morphologic forms that could potentially be defined within the population is artificial. Existence of at least two distinct shape "clusters" within the population will be suggestive of quantifiable morphological variability in shell shape. The secondary objective was to compare the results of the outline analysis of modern *A. borealis* to Pliocene *A. borealis* from Alaska. That was done to test how a completely random small sample will compare with our dataset. We had chosen a fossil, instead of recent, subset to make samples both spatially and temporally different.

This is the first comprehensive study of the shell outline variation within a single population of *Astarte borealis* based on a large number of specimens. Although *A. borealis* has been studied in comparison to other *Astarte* species (Gardner and Thompson, 1999; Ockelmann, 1958; Saleuddin, 1965, 1967, 1974; Schaefer et al., 1985; Selin, 2007; Skazina et al., 2013; Zettler M.L., 2002), comparative studies using the shell outline to determine the degree and type of variability within *A. borealis* from the same population have never been attempted. The study is important in demonstrating the potential significance of a new technique for understanding the shell shape variation in recent *A. borealis* and has significant implications for systematic studies of both recent and fossil species.

MATERIALS AND METHODS

More than 700 specimens of recent *Astarte borealis* were collected in July of 2005, along a 5.5 km stretch of gravel beach at Camden Bay, North Slope of Alaska (Figure 1).

Twenty-three fossil specimens of *Astarte borealis* used in this study were obtained on loan from the California Academy of Sciences, Department of Invertebrate Zoology, and Geology fossil collections. These specimens were collected from the Pliocene marine facies of the Milky River formation at Sandy Ridge section, Alaska Peninsula (Marincovich et al., 2002) (Figure 1). Thirteen specimens from this collection were used for the distance measurement analysis based on completeness with respect to the distance measurements needed. Eleven specimens were used for the outline analysis and were chosen based on degree of outline completeness.

All recent shells were sorted to separate left from right valves, then left valves were retained and sorted again for elimination of specimens without complete outlines. Valves with large chips or partial outlines due to breakage were removed from the study sample. A total of 641 recent left valves and 11 Pliocene specimens were used for this study. Left valves of recent specimens were chosen, with no preference over right valves, to eliminate duplication of a specimen in the quantitative analyses.

All left valves were soaked in 10% sodium hypochlorite solution for approximately 48 hours to remove periostracum, rinsed with de-ionized water, and dried. Fossil specimens were unaltered for this study. Due to limited number of specimens, both left and right valves of fossil *Astarte borealis* were used when complete outline was visible.

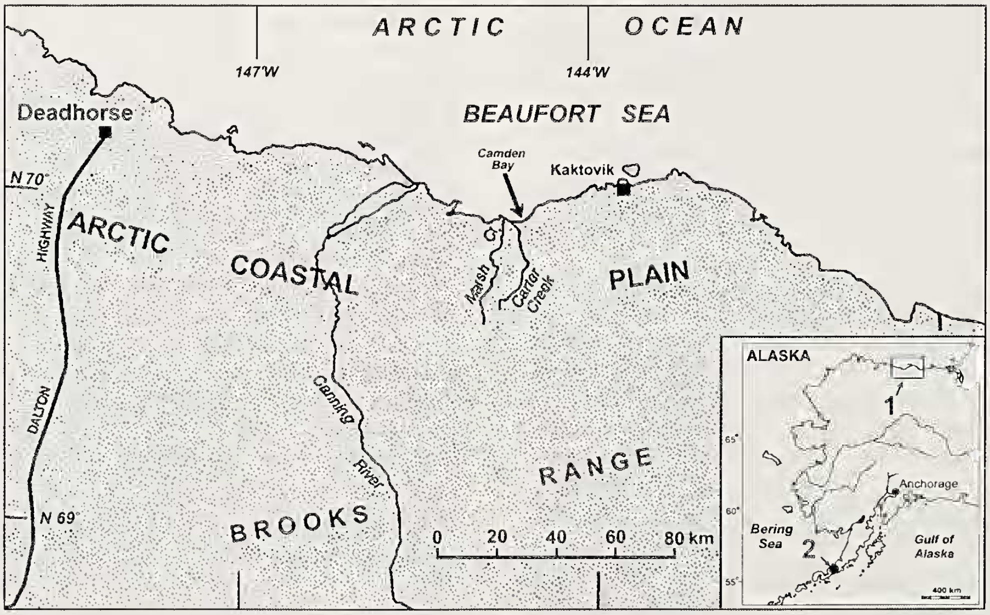

Figure 1. Map showing location of collection sites in Alaska. 1. Camden Bay, North Slope, recent *Astarte borealis*. 2. Sandy Ridge, Pliocene *A. borealis*.

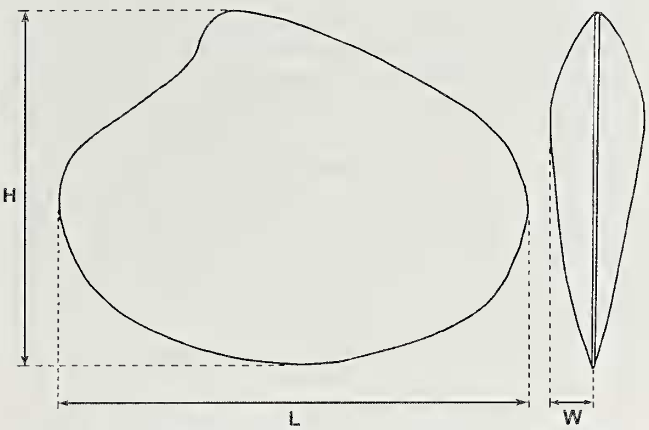

Figure 2. Outline of the shell of *Astarte borealis* showing basic measurements. H: height; L: length; W: width.

Recent shells were prepared for photography by coating the exterior surface with water-based tempera paint to remove any image interference from rust staining on the surfaces. The shells were photographed with digital camera and processed in Adobe Photoshop 7.0 to retain the outline of the image. The fossil shells had no processing prior to digital imaging, and images of any right valves were reflected geometrically to represent left valves. The last step was possible due to the equivalve nature of *A. borealis* shell (Saleuddin, 1965).

Table 1. Bivariate results for shell proportions of *Astarte borealis* in this study.

Specimen	L/H	W/H
Recent *A. borealis*	1.16	0.21
Pliocene *A.borealis*	1.12	0.24

The length to height (L/H) and width to height (W/H) ratios were calculated for one third of recent specimens that were used for outline analysis. These 225 valves were randomly selected for the distance measurements, as well as 13 fossil specimens. Regarding the measurements, length (L) is the longest distance from front to back edge; height (H) is the distance from the umbo to edge; and width (W), is the longest distance of the valve in a lateral plane across the valve (Figure 2). The linear measurements were taken using a Mitutoyo Absolute Digimatic digital caliper, and were made at a resolution of millimeters to the hundredth place (0.01 mm).

The multivariate outline analysis was performed using SHAPE ver. 1.3, a software package for Quantitative Evaluation of Biological Shapes Based on elliptical Fourier descriptors, developed by Iwata and Ukai (2002). The variation in valve outline shape, a case of a closed contour, is characterized by the Elliptic Fourier descriptors (EFDs), which are obtained by decomposing a curve into a sum of harmonically related ellipses (Kuhl and Giardina, 1982).

First, the chain code was obtained from the processed digital images, using the ChainCoder program (Iwata and Ukai, 2002). The program converts the full color image to black and white by splitting the image into three colors with gray scale, converting the image with clearest contrast to black and white. The noise is reduced and the closed contour of the valve was extracted by edge detection which is described as a chain code. Chain code

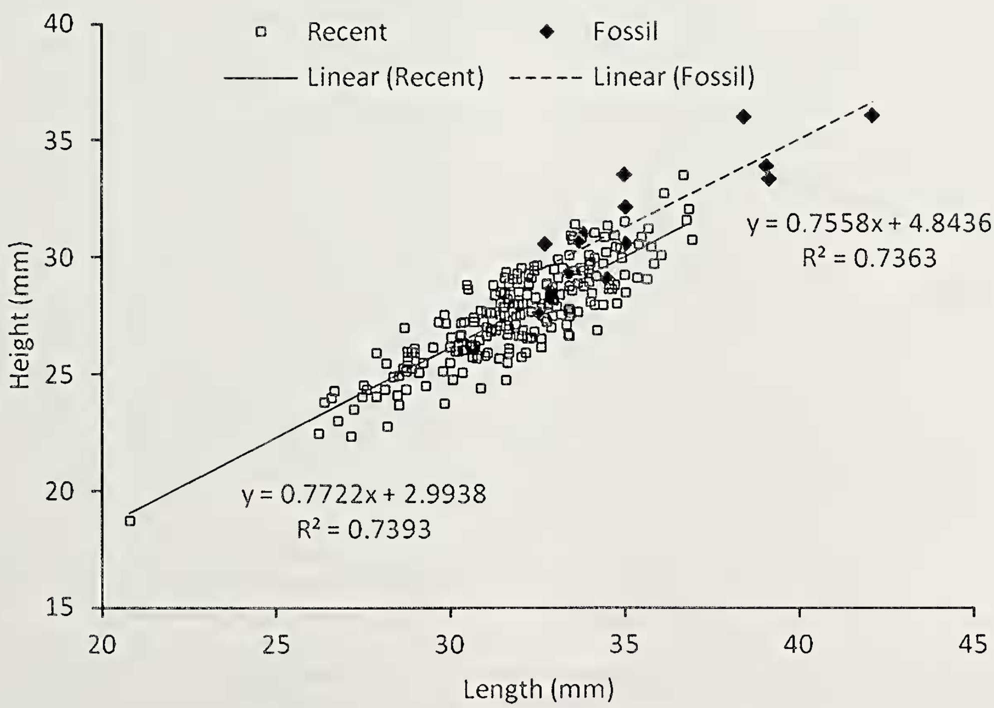

Figure 3. H/L relationship for recent and Pliocene shells of *Astarte borealis*.

is a coding system for describing geometrical information about contours using numbers from 0 to 7, indicating direction as measured counterclockwise from X axis of X–Y coordinate system to represent the position of each successive point in relation to the previous (Kuhl and Giardina, 1982). The area of each valve was also recorded.

The normalized Elliptic Fourier descriptors (EFDs) were calculated in Chc2Nef program of the SHAPE ver. 1.3 package (Iwata and Ukai, 2002). The program obtained chain code to calculate normalized EFDs following the procedures suggested by Kuhl and Giardina (1982). The EFDs are normalized to be invariant with respect to size, rotation, and starting point and are based on the first harmonic ellipse that corresponds to the contour information's first Fourier approximation. The EFDs are used to find the principal components of the shape variation. The principal component analysis of the normalized EFDs was accomplished in the PrinComp program

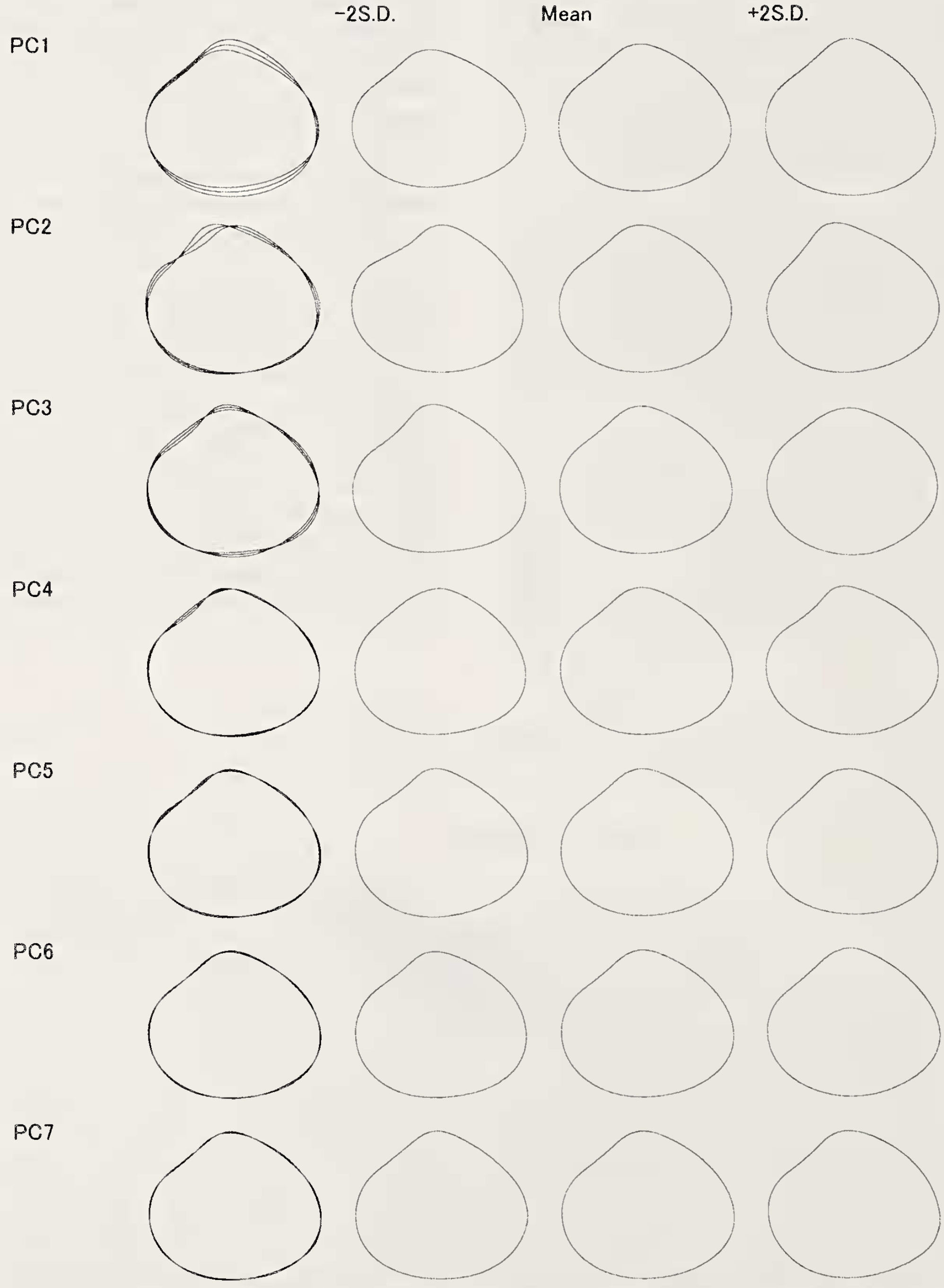

Figure 4. The PrinPrint visualization of the mean shape and the +2 and −2 standard deviation from the mean in outlines for the first 7 significant principal components. The first column shows the outlines of +2 and −2 standard deviation superimposed on the mean form.

of the SHAPE package (Iwata and Ukai, 2002) to efficiently summarize the information contained in these coefficients for easier interpretation. The principal component analysis converts a set of observed correlated variables and by means of orthogonal transformations, produces a set of values that are linearly uncorrelated variables or principal components (Rohlf and Archie, 1984). The first principal component produced has the highest possible variance with respect to the data set. Each successive principal component has the highest possible variance with respect to the preceding component. Principal components were then visualized on a chart.

The visualization of principal component analysis was done through the PrinPrint program. The program outputs the shape variation accounted for by the largest principal components. Following the procedure in Iwata and Ukai (2002), the coefficients of the EFDs are calculated such that the score for a particular principal component is equal to +2 or −2 times the standard deviation from the mean, the square root of the eigenvalue of the particular component and the scores of the remaining components are zero. The coefficients are used to execute an inverse Fourier transform and create contour shapes that are visual representations of the data. That visual output is helpful in interpreting the variation associated with each principal component.

RESULTS AND DISCUSSION

According to Selin (2007), shell proportions can be a reliable parameter for differentiating between species. The results of bivariate analysis for length to height (L/H) and width to height (W/H) ratios are shown in Table 1.

The L/H ratio is 1.16 and is comparable to the previous result of 1.15 obtained by Zettler (2001), also falling between the ratio of 1.28 obtained by Ockelmann (1958) and 1.10 by Selin (2007). The plot of height and length measurements for both recent and fossil specimens is shown on Figure 3. The correlation coefficient for height to length ratio is $R^2 = 0.7393$ for the recent (two-tailed P value is less than 0.0001) and $R^2 = 0.7363$ for the fossil specimens (two-tailed P value equals 0.0027), which suggest a very close similarity of shape between recent and fossil specimens. The outline analysis shows the areas where variability in shell outline was found as well as the proportion of variance that could be attributed to each component of variation among the population. The results of the statistical computation using the SHAPE PrinComp package and the first seven principal components are shown in Figure 4.

Principal components (PC) that represent variance in outline were calculated from the symmetric and asymmetric aspects of the shell. The PrinComp package gives results based numbers of harmonics used in the calculation; for this analysis, 77 principal components were produced. Here, only the first 10 principal components are discussed, as coefficients with small variance and covariance values are generally not important for explaining the observed morphological variations. Table 2 has first 10 PC of the recent *A. borealis* outline analysis as computed by PrinComp. The 10 PCs account for about 94.94% of the variance found in the population, and the first three PCs represent 83.21% of variance.

Table 2. Eigenvalues and contribution of principal components of *Astarte borealis* in this study.

Component	Eigenvalue	Proportion (%)	Cumulative (%)
PC 1	7.36E-04	47.4171	47.4171
PC 2	3.53E-04	22.7443	70.1614
PC 3	2.02E-04	13.0451	83.2065
PC 4	5.23E-05	3.3717	86.5782
PC 5	4.26E-05	2.7436	89.3218
PC 6	2.68E-05	1.7263	91.0482
PC 7	2.04E-05	1.3167	92.3649
PC 8	1.65E-05	1.0634	93.4282
PC 9	1.29E-05	0.8343	94.2625
PC 10	1.05E-05	0.6759	94.9384

The PCs were plotted against one another to show the concentration of variance in outline shapes (Figure 5). The greatest variation is represented by the first PC, the second greatest variation by the second PC and so forth. The distribution of variation can be visualized by plotting the PCs against one another, since the first two PCs comprise the most variation; they are plotted with the PC 1 on the x-axis and the PC 2 on the y-axis. Figure 5 shows the first and second principal components plotted with the outline contours drawn by the PrinPrint program, as well as the first and third principal components. As shown on Figure 5, PC 1 accounts for 47.42% of the variance found in the recent specimens and based on the visualization this is representative of relative shell height. From the positive to the negative standard deviations, the shell length only varies about 1% whereas the shell height varies about 15%. The positive standard deviation has a height to length ratio of 1.09, the mean shape ratio is 1.17 and the negative standard deviation has a ratio of 1.26. The mean ratio of 1.17 is consistent with the bivariate analysis results of length to height ratios (Table 1). PC 2 accounts for 22.74 % of the variance and represents the position of the umbones with respect to the central line. The mean shape has an umbo nearly on the midline, the positive deviation has the umbo quite dorsal to the midline and the negative deviation has the umbo slightly dorsal of the midline. PC 3 accounts for 13.05 % of the variance and is representative of the overall shell shape. The positive deviation has a rounded, subquadrate figure ranging to the negative valve with a compressed subtrigonal shape. This is consistent with the various descriptions available in literature. No correlations occurred when PC 2 and PC 3 were plotted against one another.

The shell outline was analyzed for 11 fossil specimens and results were plotted over the recent graphs for comparison (Figure 6). Fossil specimens fall entirely within the range of the recent specimens data. Since sample size is different, 641 recent samples and 11 Pliocene

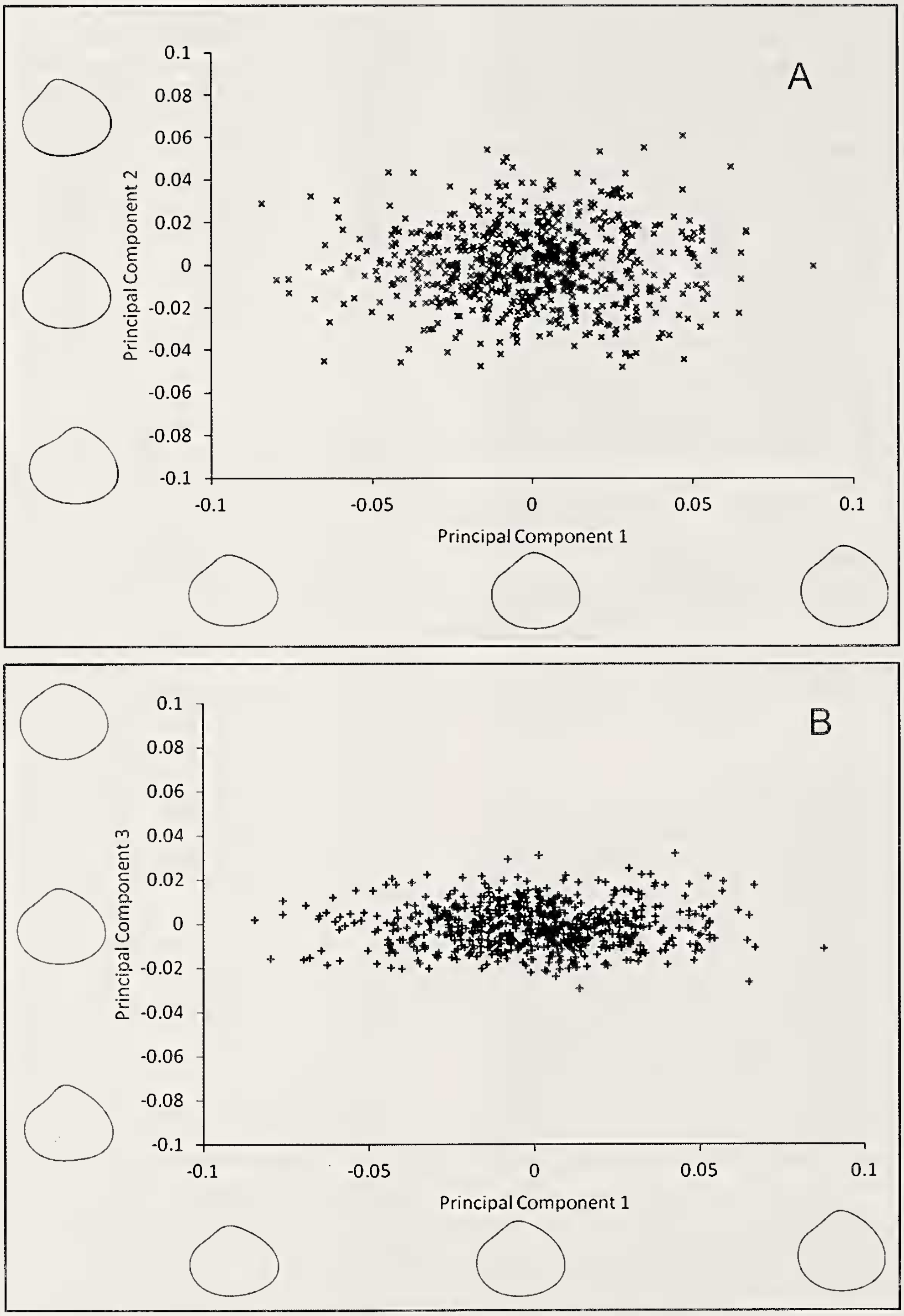

Figure 5. Plots of principle components. A. PC 1 vs. PC 2 of *Astarte borealis* with reconstructed contours. B. PC 1 vs. PC 3 of *A. borealis* with reconstructed contours.

fossil samples, one-way Kruskal and Wallis ANOVA was used to test the significant of differences ($p < 0.0001$) between the recent and fossil *A. borealis* PC 1 results (Kruskal and Wallis, 1952). This is interpreted as an indication that the morphological variability of fossils is similar to that of the recent specimens, however not a direct indicator of correspondence.

Existing paleontological descriptions of fossil and recent species of *Astarte* suggest great similarity in shape among species. Use of elliptical Fourier analysis is an attempt to show that the outline of fossil clams match the modern and fall into the same distribution.

Morphologic variability of shell is one of the main criteria used for identification of fossil species of bivalve

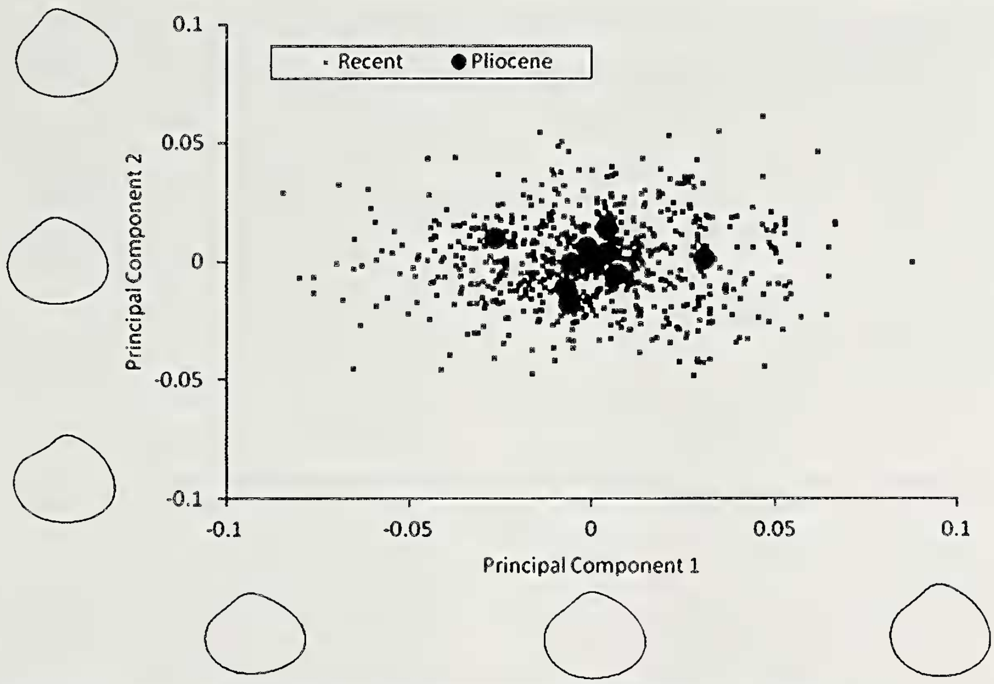

Figure 6. Principal Component 1 vs. Principal Component 2 for recent and Pliocene *A. borealis*.

mollusks. It is, therefore, important to understand the limits of morphologic variability within the same species and population of bivalve mollusks, particularly the ones lacking prominent and diagnostic morphologic characteristics.

Outline shape analysis clearly indicates that *Astarte borealis* has a high morphologieal variability at the species level and even within a single population, which can be a potential source of confusion during species identification, especially with the use of qualitative descriptive parameters for shell shape. The bivariate comparison analysis between Plioeene and recent populations shows that there is continuity in the species and that the variation has been a characteristic of the species for a long time. The multivariate outline analysis indicates that the intraspecific variation is based upon a common shape that grades into other shapes evenly or a correlated population centered on a common "central" form. This study does not support the idea that the variation within certain species is a continuum between two end forms. For the studied population of *Astarte borealis*, allocation to forms and varieties would be at best problematic. Separation of fossil species in the absence of a large specimens sample should not be based solely on the shell outline and should include other diagnostie characteristics, such as external sculpture, details of the hinge, and features of the shell margin.

The study of geographical distribution of species by Zettler (2002) emphasized and confirms the polymorphism of *A. borealis*. The polymorphism of the species has been attributed to non-pelagic reproduction that causes the eggs to attach to the substrate near the parents (Bernard, 1979; Ockelman, 1958). Since there is a lack of diversity in reproduction due to limited genetic mixing and a greater chance of isolation in the population, the variation could accelerate depending upon environmental conditions such as substrate composition, salinity, temperature, and nutrients. The slight differences in environment may influence the changes in the shell height to length ratio or perhaps the ventral margin that affect a certain portion of the population but do not separate it from the species. Changes can probably be detected in shell thickness and overall size, which could be a function of water temperature. That, in turn would be a function of changing climate.

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